Altered Recovery of Macular Function after Bleaching in Stargardt's Disease-Fundus Flavimaculatus: Pattern VEP Evidence

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Purpose. To evaluate recovery of pattern visual evoked potentials (VEPs) after macular bleaching in patients with Stargardt's disease-fundus flavimaculatus (STD/FF).

Methods. Sixteen unrelated patients with STD/FF (age, 26–52 years; visual acuity, 0.2–1.0; phenotype I, $n = 6$; phenotype II, $n = 8$; or phenotype III, $n = 2$) and 15 age-matched control subjects were evaluated. VEPs were recorded in response to counterphased (two reversals per second) checkerboards (check size, 15 minutes; mean luminance, 80 cd/m^2; contrast, 80%; stimulus field size, 18°) in baseline condition and at 20, 40, and 60 seconds after a 30-second exposure to a bleaching light (3.58 log photopic trolands), presented to the central (6° field) retina. In all patients, macular focal electroretinograms (FERGs) to an 18° uniform field, flickering at 41 Hz, were also recorded in separate sessions.

Results. At every postbleaching time, VEPs were delayed and suppressed in amplitude, compared with prebleaching values, in both patients and control subjects. However, the amount of delay and suppression was, on average, more pronounced ($P < 0.001$) in patients than in control subjects. This difference was not accounted for by eccentric fixation in patients ($n = 8$) with central scotoma and was still substantial when only patients ($n = 8$) with normal visual field and acuity were considered. In individual patients, baseline FERG amplitudes correlated ($r = -0.6$, $P < 0.01$) with the suppression of VEP amplitude at 40-seconds after bleaching.

Conclusions. The results indicate an altered recovery of pattern VEPs after macular bleaching in STD/FF and suggest adaptation abnormalities in macular cone photoreceptors, occurring at disease stages with relatively preserved central visual field and acuity. (*Invest Ophthalmol Vis Sci.* 2002;43:2741–2748)

Stargardt disease (STD) is the most common hereditary recessive macular dystrophy[1] characterized by juvenile to young-adult onset, central visual impairment, and progressive bilateral atrophy of the macula and retinal pigment epithelium (RPE), with a frequent appearance of orange-yellow flecks distributed around the macula and/or the midperipheral retina.[2] A clinically similar retinal disorder, fundus flavimaculatus (FF), often displays later ages of onset and slower progression. Previous, extensive clinical studies[2–4] and more recent studies evaluating genotype-phenotype correlations,[5] suggest that STD and FF may not represent different genetic disorders.

There are many reports showing that the function of both macular and peripheral cones, as well as rod function, can be abnormal in STD/FF.[6–8] There is also evidence that STD/FF is associated with characteristic abnormalities of dark adaptation,[9] involving delayed recovery after bleaching to baseline sensitivity of the last branch of the adaptation curve. Similar abnormalities in rod dark adaptation have been recently found by Aleman et al.[10] in patients with known genotype. Although rod adaptation kinetics has been found to be abnormal in STD/FF, cone adaptation has not been investigated in detail. For instance, cone kinetics were not reported in the study by Fishman et al.[9]

An alternative approach that has been used to test recovery of cone function after bleaching is the test of macular recovery after photostress.[11] In one psychophysical version of the test, the recovery in visual acuity after the delivery of a bright-adapting light (bleaching or photostress) to the macula, is evaluated. The time needed to reach the baseline acuity before bleaching is measured.[11,12] In another electrophysiological version of the photostress test, VEPs in response to contrast-reversing checkerboards are measured as the outcome variable.[13–15] Bleaching induces VEP changes consisting in an increased time-to-peak and a decreased amplitude of the major positive component (i.e., P100) of the pattern reversal response. The recovery in response parameters can be determined objectively and quantitatively at different postexposure times, in both normal and diseased eyes.[15,16]

Evaluating the recovery of spatial vision after bleaching, either by visual acuity or VEPs, may provide a sensitive test of the adaptation of the cone system. The visual acuity recovery test result has been found to be significantly altered in several macular disorders,[11] including the early stages of age-related macular degeneration.[12,17] The VEP recovery test has been reported to be sensitive, although not specific, in detecting early macular dysfunction with different underlying pathophysiologies.[16]

The purpose of the present study was to evaluate in patients with STD/FF the recovery of macular function after bleaching by pattern-reversal VEPs and to correlate changes in the VEP parameters' recovery with other clinical (visual acuity) and functional (focal electroretinogram) indicators of macular disease in the same patients.

Subjects and Methods

Patients

Sixteen unrelated patients (6 men, 10 women) with a clinical diagnosis of STD/FF were included in the study. Patients' ages ranged between

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TABLE 1. Clinical Findings

Patient	Age (y)	Eye	Phenotype	Visual Acuity	Silent Choroid (Macula)	Silent Choroid (Mid-periphery)	Macular Lesion	Flecks†	Central Scotoma*
FA	29	R	I	0.3	−	−	Atrophic lesion	Perifoveal flecks	+
CI	50	R	I	0.4	−	−	Atrophic lesion	Perifoveal flecks	+
NO	31	L	I	0.8	−	−		Perifoveal flecks	−
BA	21	R	I	0.8	−	−		Perifoveal flecks	−
SA	31	R	I	0.1	−	−	Atrophic lesion	Parafoveal flecks	+
PI	21	L	I	0.1	−	−	Atrophic lesion	Parafoveal flecks	+
BI	49	L	II	1.0	+	−		Diffuse flecks	−
LD	52	L	II	1.0	+	−		Diffuse flecks	−
NE	49	R	II	0.1	+	+	Atrophic lesion	Diffuse flecks	+
MZ	52	R	II	0.1	+	+	Atrophic lesion	Diffuse flecks	+
BR	29	R	II	1.0	+	−		Diffuse flecks	−
UN	50	L	II	1.0	+	−		Diffuse flecks	−
OF	50	L	II	0.6	+	−	Bullseye lesion	Diffuse flecks	−
RE	26	R	II	0.8	+	−	Bullseye lesion	Diffuse flecks	−
IG	26	L	III	0.1	−	−	Atrophic lesion		+
ES	50	R	III	0.1	−	−	Atrophic lesion		+

* More than two points with more than 5 dB loss within the central 10° in a Humphrey 30-2 threshold test (Humphrey Instruments, Dublin, CA).

† Fluorescein angiographic hyperfluorescence.

21 and 56 years (mean, 38.5 ± 12.5 years). All patients underwent general clinical and ophthalmic examinations. These included best corrected visual acuity with a Snellen projection chart, slit lamp examination of the anterior and posterior segments, direct and indirect ophthalmoscopy and retinal biomicroscopy, and applanation tonometry. Fluorescein angiography, visual field testing (Goldmann and Humphrey Statpac 30-2; Humphrey Field Analyzer; Humphrey Instruments, Dublin, CA) and ganzfeld electroretinography (according to International Society for Clinical Electrophysiology of Vision [ISCEV] standards[18]) were also performed in all patients. STD/FF diagnosis was based on clinical history, ophthalmic examination, fluorescein angiography, and ganzfeld electroretinography. All patients met the following inclusion criteria: no history of concomitant neurologic or metabolic diseases; no concomitant ocular disorders (including optical media opacity, optic neuritis, amblyopia, and glaucoma); best corrected visual acuity of 0.1 or more, refractive errors less than ±3 D (spherical equivalent); stable central fixation, as evaluated by the Visuskope (Heine, Germany), and no history of medication that can affect macular function (e.g., chloroquine). None of the patients enrolled in the study had clinically detectable optic nerve pallor. Clinical and demographic data of individual patients are reported in Table 1. At clinical examination, six patients showed phenotype I, according to the classification used by Fishman et al.,[5] with an atrophic foveal lesion, localized perifoveal white flecks, and no absence of choroidal circulation (silent choroid). Eight patients had phenotype II, with more diffuse white flecks and a silent choroid in the macula and/or midperiphery, and two patients had phenotype III with atrophic-appearing changes in the RPE. In the latter two patients, STD/FF diagnosis was supported by fluorescein angiograms performed 8 years before enrollment in the study, and showing, at that time, many confluent flecks in the macular region. Visual acuity ranged 0.1 to 0.8 in patients with phenotype I, 0.1 to 1.0 in those with phenotype II, and 0.1 in those with phenotype III. Ganzfeld rod and cone-mediated electroretinograms were normal in 10 patients, and only mildly abnormal (<30% amplitude reduction of b-wave, <5 ms time-to-peak b-wave delay) in the remaining 6 patients. Eight patients (Table 1) had a central scotoma, as defined by the presence of more than two points with a sensitivity loss of more than 5 dB within the central 10° in the field analyzer 30-2 threshold test.

Fifteen normal control subjects (5 men, 10 women), whose age distribution (mean, 37 ± 11 years; range, 20–58) was comparable with that of the patients, underwent VEP recordings after bleaching. All subjects had normal general and ophthalmic examination, a visual acuity of 1.0 in both eyes, a normal field (field analyzer program 30-2 mean deviation more than −0.5, absence of points with abnormal sensitivity), and no family history of retinal degenerative disease. Another, independent group of 12 normal subjects (four men, eight women, mean age, 36 ± 10; range, 32–56) provided normative data for focal electroretinogram recordings.

Informed consent was obtained from each patient and control subject before his or her inclusion in the study and after the goals and procedures of the research were fully explained. The research adhered to the tenets of the Declaration of Helsinki, and appropriate institutional review board approval was obtained.

Electrophysiological Methods

VEPs in Baseline Condition and after Bleaching. Visual stimuli consisted of checkerboard patterns (a single check edge subtended 15 minutes of visual arc; contrast, 80%; mean luminance, 80 cd/m^2) generated on a monitor subtending 18° and reversed in contrast at the rate of two reversals per second. The stimulation was monocular, with full occlusion of the fellow eye. To maintain stable fixation, a small red target (0.5°) was placed in the center of the stimulation field. VEPs were recorded by cup-shaped Ag/AgCl electrodes placed over the scalp 2 cm above the inion (Oz), with the reference in Fpz and the ground in the left arm. The interelectrode resistance was kept below 3 kΩ. VEP signals were amplified (gain 20,000), filtered (band-pass 1–100 Hz, −6 dB/octave), sampled with 12-bit resolution, and averaged with automatic artifact rejection.

The test of recovery of pattern VEPs after macular bleaching was performed according to a published protocol.[15,16,19,20] The procedure involved three steps: recording of baseline VEP, bleaching of the central retina, and recording of VEPs at predetermined times after bleaching. Baseline VEP recording was performed as follows. Two VEP responses (analysis time 500 ms, averaged over 100 stimulus periods) were obtained at the beginning of the session, and the loss in recording time due to artifacts was noted.[15] The time-to-peak (in milliseconds) and peak-to-peak amplitude (in microvolts) of major VEP components (i.e., N75, P100, and N145) were measured. After this preliminary trial, a VEP recording was obtained by reducing the number of averaged sweeps to 40 per record (recording time for each trace, 20 seconds). The response was accepted only if no more than two individual sweeps were discarded because of artifacts. The resultant waveform was kept on display on the computer screen and considered the baseline VEP. Variability of this baseline VEP was then estimated by taking six consecutive recordings of 40 events each. Based on these measurements, the SD of the P100 time-to-peak and the coefficient of variation of the N75-to-P100 amplitude were determined.

TABLE 2. Electrophysiological Data in Patients, at Baseline and after Bleaching

Patient	Eye	Focal ERG Amplitude (μV)	Phase (Deg)	VEP P100 Time-to-Peak (ms)				VEP N75–P100 Amplitude (μV)			
				Basal	20 sec	40 sec	60 sec	Basal	20 sec	40 sec	60 sec
FA	R	0.34	−227.1	129	152	157	157	2.6	0.8	1.5	2.0
CI	R	0.18	−141.2	137	160	154	142	1.9	1.3	1.1	1.2
NO	L	0.53	−81.9	132	153	148	139	2.8	1.3	1.4	2.7
BA	R	0.42	−53.2	139	156	156	148	1.6	0.8	1.2	1.2
SA	R	0.15	−148.5	122	129	125	126	6.0	3.1	1.9	3.3
PI	L	0.25	−173.0	112	133	131	125	4.3	2.2	2.1	1.9
BI	L	0.11	−275.0	129	160	160	157	3.6	1.9	1.9	1.9
LD	L	0.16	−320.0	141	172	172	162	4.8	1.3	1.3	1.8
NE	R	0.49	−80.2	133	152	148	145	3.6	1.9	1.2	1.6
MZ	R	0.50	−144.0	134	148	152	144	1.9	0.4	1.2	1.3
BR	R	1.39	−51.7	103	129	123	118	10.3	5.0	8.7	7.4
UN	L	1.63	−227.1	109	129	123	121	10.0	3.7	6.5	9.0
OF	L	0.84	−181.6	107	113	111	110	12.7	7.9	9.2	10.5
RE	R	0.79	−168.3	109	123	121	119	8.8	4.1	7.0	3.7
IG	L	0.47	−192.4	126	141	133	133	4.6	2.3	2.6	3.5
ES	R	0.17	−143.4	126	140	138	138	6.5	2.6	2.6	2.6
Mean		2.58	−33	103.1	114.3	108.4	106.0	9.18	7.33	7.78	8.03
95% confidence Limits of controls		0.50*	−89*	110.1†	120.5†	115.4†	113.7†	4.53*	3.45*	4.68*	3.38*

* Lower confidence limit.
† Upper confidence limit.

Bleaching of the central retina was performed for 30 seconds by means of a circular diffusing surface retroilluminated by a 200-W lamp. The subjects fixated the center of the circular surface, with natural pupils, from a distance of 20 cm. The bleaching field subtended 6° at the subjects' viewing distance. Retinal luminance during bleaching was 3.58 log photopic trolands, estimated to bleach approximately 20% of the cone photopigment.[21] In normal subjects, bleaching usually produced a central relative scotoma of 6° in diameter. During the procedure, the pupil diameter, measured by an observer by means of a ruler and a magnifying lens, decreased from the prebleaching value (mean, 3.5 ± 0.5 mm in both control subjects and patients) to 2 ± 0.4 mm in control subjects and 2 ± 0.3 mm in patients. At 20 seconds after bleaching, the pupil diameter had already recovered to the prebleaching value in all control subjects and patients and did not change significantly during the recording time.

Immediately after the end of bleaching, the subject started to fixate the center of the pattern stimulus (in correspondence with the fixation target) and VEP recording was initiated. The small red target was perceived by all subjects and patients, notwithstanding the presence of a subjective scotoma. Three consecutive recordings were obtained, each 20 seconds in length (40 sweeps/record) and stored in the computer and shown on the screen. Recording was further pursued until the response waveform was identical with that obtained at baseline. However, the experimental time corresponding to the full recovery of the VEP to the prebleaching waveform was not determined with precision, because of the relatively poor temporal resolution of the method. P100 time-to-peak and N75-to-P100 amplitude in the three postbleaching recordings were measured and taken as the main outcome parameters.

Because fixation in patients with reduced visual acuity may have been eccentric in the recordings after bleaching,[22] control experiments were performed in normal subjects to evaluate the effects of eccentric fixation on VEP response recovery after bleaching. VEPs were recorded while the subjects fixated an eccentric target, placed

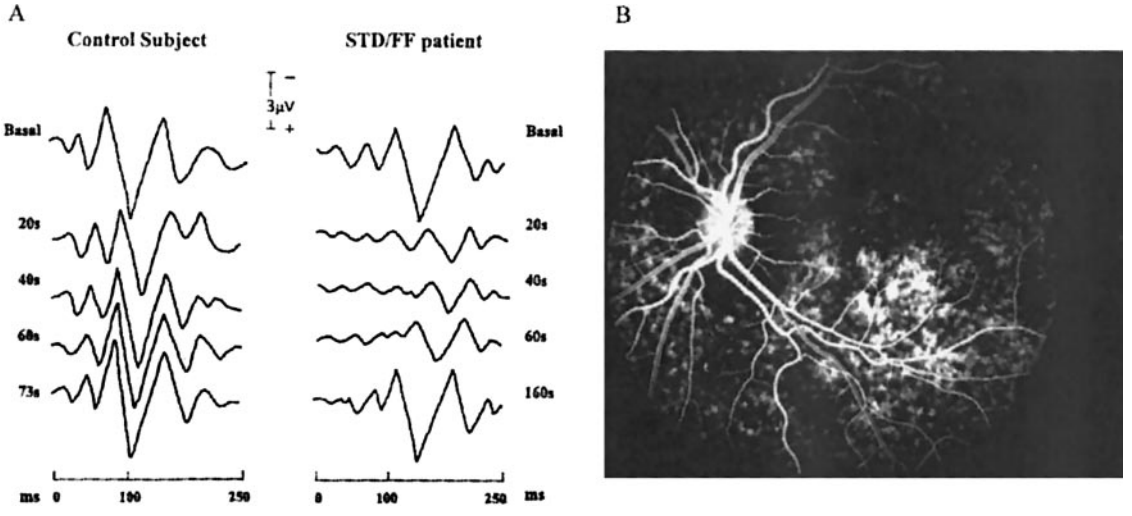

FIGURE 1. (**A**) Representative examples of VEP recordings at baseline and at various times after bleaching obtained from one control subject and one patient with STD/FF (patient LD in Table 1). The *bottom* trace is of the recording taken at the time of full response recovery (i.e., recovery time). (**B**) Fluorescein angiogram of the same patient whose VEPs are depicted in (**A**), showing pathologic hyperfluorescence due to flecks in the posterior pole and midperiphery.

10° temporally on the monitor (so that the nasal visual field was stimulated), both at baseline and after the corresponding temporal retinal area was bleached. It was verified that bleaching produced a relative scotoma of 6°, perceived in the nasal visual field. Immediately after bleaching, the recovery of VEP parameters was determined with the method described.

In each subject or patient, the signal-to-noise ratio (SNR) of the VEP response was assessed by measuring a noise response while the subject fixated an unmodulated field of the same mean luminance as the stimulus. A noise record of 40 and another of 160 events (i.e., the number of events corresponding to the total averaging process included in the experiment) were obtained. Both recordings were performed immediately after the end of the bleaching protocol. The two noise peak-to-peak amplitudes were measured in a temporal window corresponding to that at which the response component of interest (i.e., N75–P100) was expected to peak. SNRs for this component were determined, either in the short or long averaging record, by dividing the peak amplitude of the component by the amplitude of noise in the corresponding temporal window. In all subjects and patients, the VEP SNRs, determined in both ways, were 2.8 or more in all the steps of the experimental procedure.

Focal Electroretinograms. The methods used to obtain focal electroretinograms (FERGs) have been described in detail in a recently published study.[23] Briefly, FERGs were monocularly recorded by skin electrodes in response to an 18° uniform field, flickered sinusoidally at 41 Hz (mean luminance: 80 cd/m^2; modulation depth: 93.5%) and surrounded by a ganzfeld of the same mean luminance. Pupils were pharmacologically dilated to 8 to 9 mm. Acquired signals underwent Fourier analysis to isolate the fundamental harmonic component (i.e., that at the frequency of 41 Hz), with measurement of the component's amplitude (in microvolts) and phase (in degrees). Examples of FERG waveforms obtained with the present technique have been shown elsewhere.[16,23]

Statistical Analysis

One eye, randomly selected for each patient and control subject, was evaluated. VEP results from patients and control subjects were compared by a two-way analysis of variance, after normalization of both time-to-peak (taken as the difference in milliseconds from the prebleaching value at various times after the bleaching) and amplitude taken as the fraction of amplitude suppression from the baseline value at various times after bleaching: $[(Amp_{postbleach} - Amp_{baseline})/Amp_{baseline}]$. In the analysis, group (control subjects versus patients) was the between-subjects factor, and experimental time (i.e., the postbleaching recording times of 20, 40, and 60 seconds), the within-subjects factor. The P100 time-to-peak and the amplitude of the N75-to-P100 complex were separately analyzed. Pearson's correlation was used to correlate visual acuity and FERG amplitudes with VEP after bleaching parameters. In all the analyses, a two-tailed $P < 0.05$ was considered statistically significant.

Results

Individual VEPs before and after bleaching, as well as FERG results for each patient are reported in Table 2. In the same table, normative values (mean and 95% confidence limits) for each VEP and FERG parameter are also reported. Eleven of 16 patients had abnormally delayed and/or reduced baseline VEPs. Fourteen of the 16 patients had abnormally reduced and/or delayed FERGs. Regarding the variability of baseline VEP parameters, the SD of P100 time-to-peak ranged from 0.5 to 2 ms (mean, 1.2) in normal subjects and from 1 to 4 ms (mean, 2) in patients. Coefficient of variation of amplitude ranged 2% to 5% (mean, 3.4%) in control subjects and 5% to 10% (mean, 7.1%) in patients.

Examples of VEP waveforms recorded in a control subject and a patient with STD/FF, at baseline and various times after bleaching, are shown (patient LD in Table 1 and 2, visual acuity 1.0 in the tested eye) in Figure 1A. Figure 1B shows a fluorescein angiogram of the same patient whose VEPs are depicted in Figure 1A. It can be seen that, in the patient with STD/FF, there was a response amplitude suppression and a delay, after bleaching, that was substantially greater than that observed in the control subject. In addition, full postbleaching recovery of the VEP waveform was obtained in the patient only 160 seconds after bleaching, compared with the 73 seconds estimated for the normal control subject.

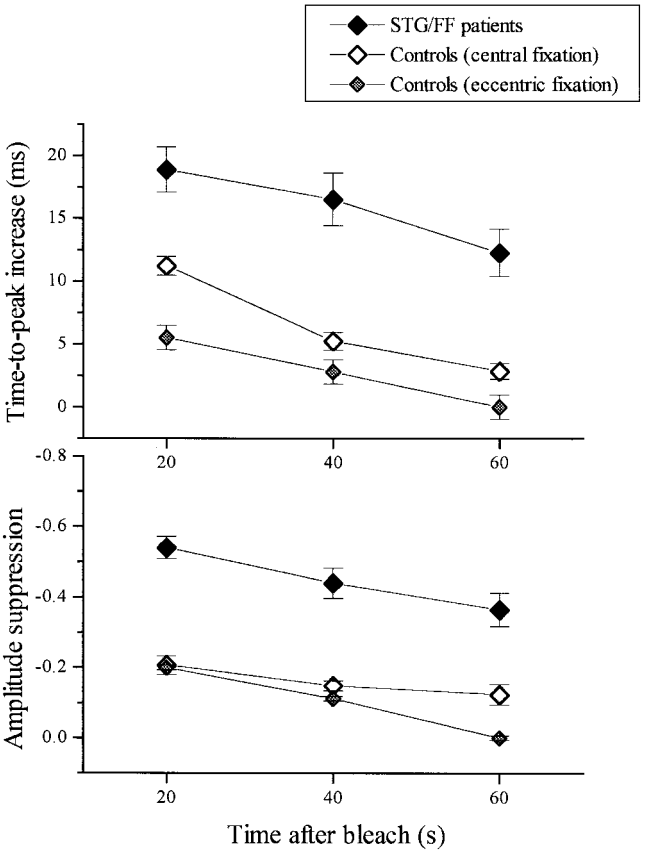

Figure 2. Mean (±SE) relative changes in VEP parameters (increase in P100 time-to-peak from baseline, *top*; percentage of N75-to-P100 amplitude suppression from baseline; *bottom*) recorded in control subjects and patients with STD/FF at 20, 40, and 60 seconds after bleaching. Data of control subjects were obtained for both central and eccentric fixation.

At 20, 40, and 60 seconds after bleaching, the average P100 time-to-peak and N75-to-P100 amplitude of patients were respectively delayed and reduced, compared with both baseline data and the data of control subjects obtained under central or eccentric fixation. The normalized mean (± SE) VEP P100 time-to-peak and N75-to-P100 amplitude recorded from control subjects (during both central and eccentric fixation) and patients at the three experimental times after bleaching, are presented in Figure 2. A two-way ANOVA performed on the data obtained from patients and control subjects under central fixation conditions gave the following results: P100 time-to-peak, significant effect of group (i.e., difference between control subjects and patients, $F_{(1,29)}$: 50.5, $P < 0.001$) and time (i.e., mean changes across different experimental times, $F_{(3,87)}$: 107.8, $P < 0.0001$), and significant interaction of group by time (i.e., group differences were dependent on the experimental time, $F_{(3,87)}$: 16.255, $P < 0.001$); N75-to-P100 amplitude, significant effect of group ($F_{(1,29)}$: 27.3, $P < 0.001$) and time

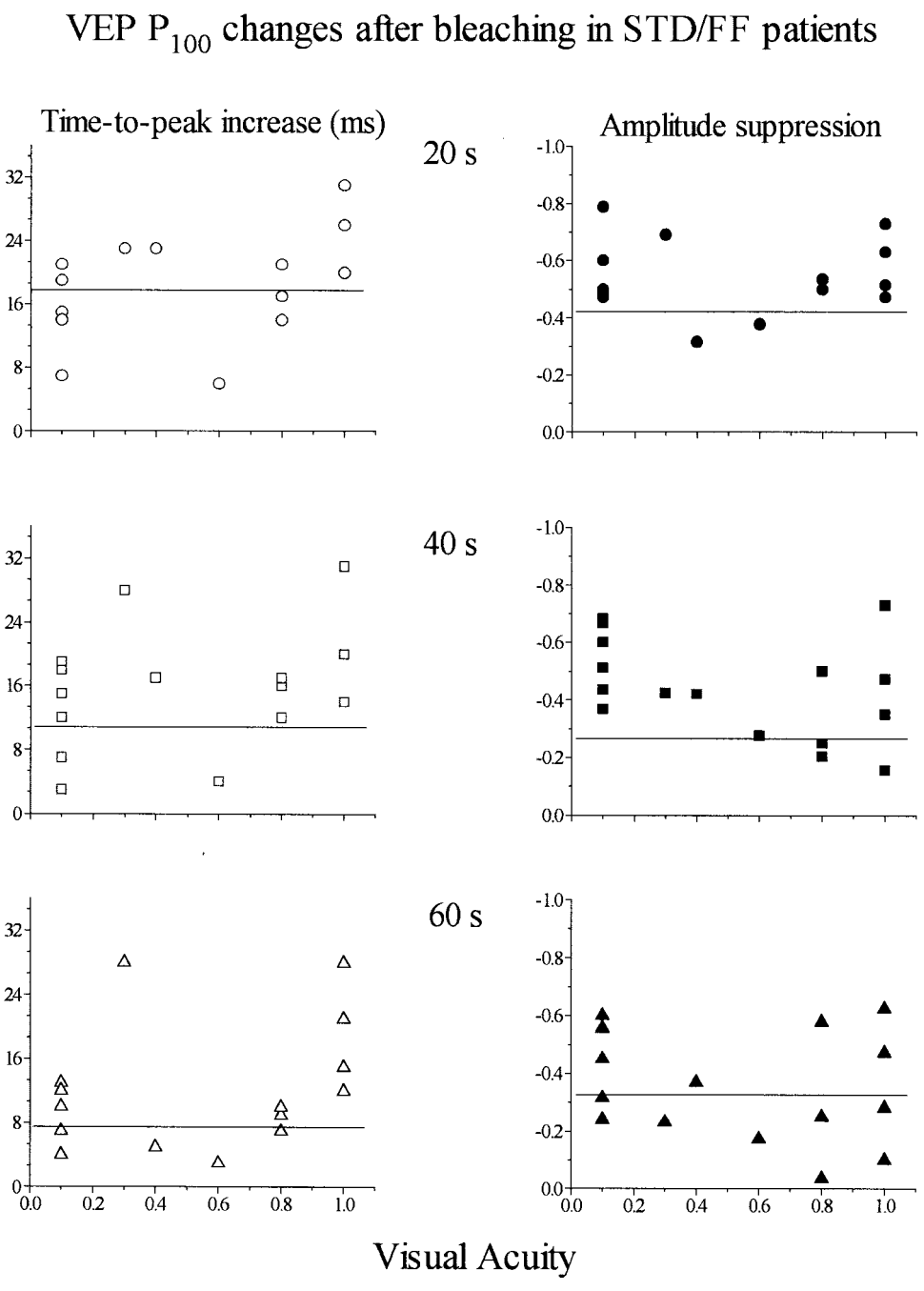

FIGURE 3. Relative changes in VEP parameters (increase in P100 time-to-peak from baseline, *left*; percentage of N75-to-P100 amplitude suppression from baseline, *right*) of individual patients plotted as a function of corresponding visual acuities. Data are shown separately for 20 (*top*), 40 (*middle*), and 60 (*bottom*) seconds after bleaching. *Horizontal lines*: 95% confidence limits of normal values.

($F_{(3,87)}$: 39.3, $P < 0.001$), and no significant interaction of group by time ($F_{(3,87)}$: 1.82, $P = 0.15$). A two-way ANOVA performed on the data obtained from patients and control subjects under eccentric fixation provided results essentially identical with those obtained by analyzing the data acquired under central fixation.

In Figure 3, the changes in VEP time-to-peak and amplitude in individual patients at different times after bleaching are plotted as a function of the corresponding visual acuities. Horizontal lines in the plots indicate the upper 95% confidence limits of normal values. No significant correlations were observed between any of the VEP parameters and visual acuity. It can be noted from inspection of the plots in Figure 3 that substantially abnormal VEP time-to-peak and amplitude changes were observed at every postbleaching time in patients with normal or relatively preserved visual acuity (>0.5). These changes are similar in magnitude to those in patients with low acuity. To further explore this aspect, VEP changes after bleaching were also compared between patients with or without central scotoma in the field analysis program 30-2 test. Figure 4 shows changes in VEP time-to-peak and amplitude parameters obtained in patients in the two subgroups at the different times after bleaching. The scatter of normal control data is also shown for comparison. The figure shows that both VEP parameters were altered to a similar extent, compared with control distributions, in patients with or without central scotoma.

FERG amplitudes in patients were negatively correlated with the corresponding amount of VEP amplitude suppression at 40 ($r = -0.63$, $P < 0.01$) and 60 ($r = -0.51$, $P < 0.05$) seconds after bleaching. In Figure 5 the percentage of suppression of VEP amplitude recorded in individual patients at 40

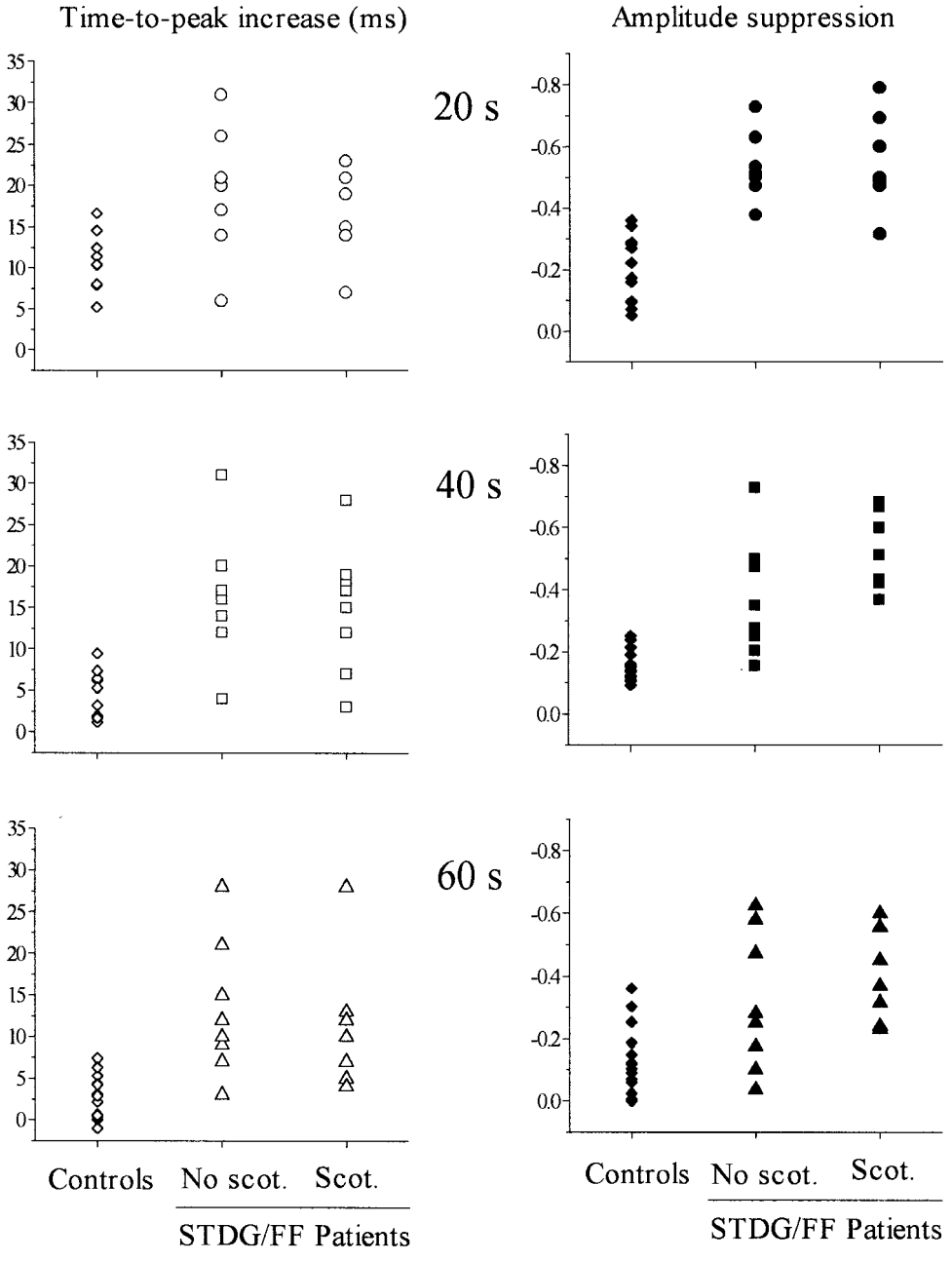

FIGURE 4. Relative changes in VEP parameters (increase in P100 time-to-peak from baseline, *left*; percentage of N75-to-P100 amplitude suppression from baseline, *right*) recorded in individual control subjects, patients with STD/FF without central scotoma (No scot.), and patients with STD/FF with central scotoma (Scot.) Data are shown separately for 20 (*top*), 40 (*middle*), and 60 (*bottom*) seconds after bleaching.

seconds after bleaching is plotted as a function of corresponding FERG amplitudes. It can be noted that greater VEP amplitude suppression tended to be associated with smaller FERG amplitude, indicating that, in individual patients, VEP amplitude changes after bleaching were at least in part predicted by the FERG amplitudes recorded from the central 18° of the retina under baseline conditions.

DISCUSSION

The results of this study indicate that, on average, the suppressive effect of bleaching on pattern VEP time-to-peak and amplitude was largely more pronounced in patients with STD/FF than in age-matched control subjects. The effect was also substantial in patients who had normal or near normal acuity and no central scotoma, indicating that it was not a mere consequence of poor central visual function at baseline. Eccentric fixation in patients with reduced acuity[22] may have been a source of artifacts, especially during the recovery phase of the VEP recordings. However, data obtained from normal subjects under eccentric fixation, when compared with patients' results, ruled out this possibility. It should be noted that VEP recordings before and after bleaching were performed with natural pupils, and therefore the bleaching-induced myosis may have contributed to the slowing of response time-to-peak in both patients and control subjects.[24] However, because pupillary changes did not differ significantly between groups, they cannot explain the differences in increase in postbleaching time-to-peak or recovery rate observed between patients and control subjects.

The abnormalities in the VEP postbleaching test observed in patients with STD/FF probably reflect an impairment of macular cone photoreceptor adaptation, due to altered photopigment density and regeneration. Although the genotype was not known in any of the tested patients, it is highly probable that, in at least some of them, the disease was the result of a

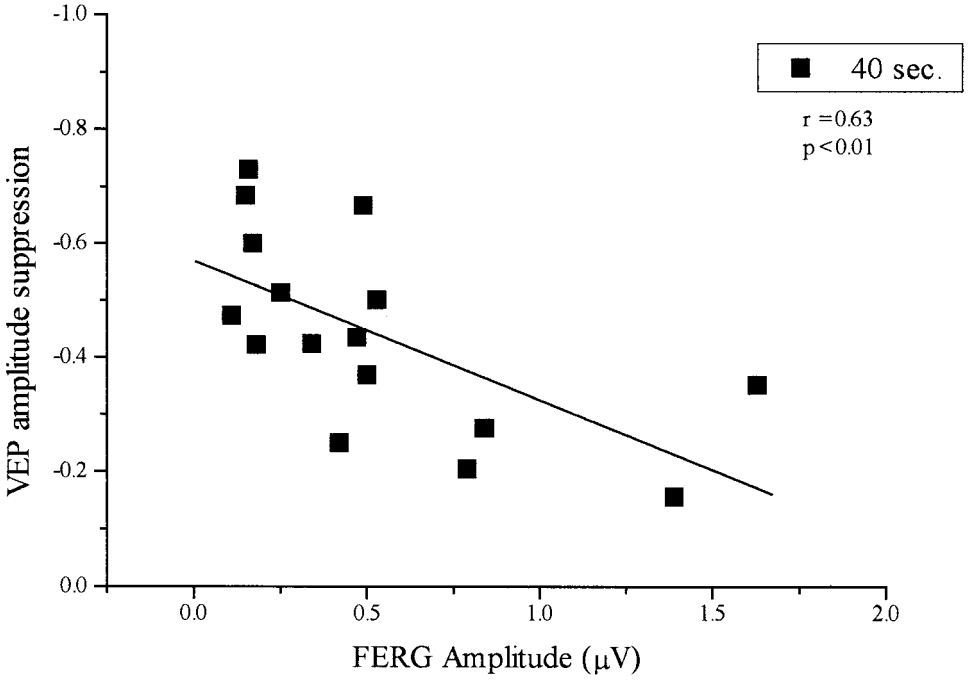

FIGURE 5. VEP percentage of N75-to-P100 amplitude suppression from baseline, recorded in patients with STG/FF at 40 seconds after bleaching, plotted as a function of corresponding FERG amplitudes. Regression line is fitted to the data points.

mutation in the gene encoding a photoreceptor gene-specific adenosine triphosphate (ATP)-binding cassette (ABC) transporter (*ABCR*), mapping to chromosome 1p13-p21, which has been found to be the causative gene in STD/FF.[25] The *ABCR* gene is expressed exclusively and at high levels in the retina, in both rod and cone photoreceptors.[26] A recent study by Weng et al.,[27] investigating the molecular mechanisms underlying photoreceptor degeneration in *ABCR* mutation-induced STD, proposed that photoreceptors die as a consequence of poisoning of the RPE by accumulation of lipofuscin and loss of the RPE support role. An experimental study in mice heterozygous for a null mutation of the *ABCR* gene[28] found lipofuscin accumulation and delayed dark adaptation similar to that observed in STD/FF. Delayed dark adaptation was also observed in patients with cone–rod dystrophy associated with mutations in the *ABCR* gene.[29] The mutation-induced disease may affect cone photoreceptors at relatively early stages, either directly or indirectly, through altered retinoid recycling.

It has been also reported that foveal cone pigment density is greatly reduced in patients with STD, and pigment regeneration can be abnormally delayed.[30] These data support the hypothesis that the enhanced effect of bleaching on VEP time-to-peak and amplitude in patients is a direct result of abnormal cone pigment density and regeneration. This could explain the delayed response recovery observed even in patients with well-preserved visual acuity. Abnormalities involving the inner retina may also have contributed to the enhanced losses and delayed recovery after bleaching found in VEP responses of patients. Indeed, degeneration of retinal ganglion cells and hyalinization of the vessels of the inner retina, which were more pronounced in the posterior pole, have been described in a 62-year-old STD/FF donor eye.[31] However, in individual patients, the amount of amplitude loss of the FERG, an assay of outer retinal function,[32] significantly predicted the corresponding amount of postbleaching VEP amplitude losses. It is possible that the experimental conditions (i.e., background and stimulus mean luminance viewed through a dilated pupil) under which the FERG was recorded may have produced a significant bleach of cone photopigment, thus contributing to the correlation with VEP amplitude changes after bleaching. This correlation, obtained from the same stimulated area, further supports the outer retina (i.e., photoreceptors and bipolar cells) as being the main locus of the bleaching-induced dysfunction.

An explanation for the substantial postbleaching changes of cone function observed, even in patients with STD/FF with preserved visual acuity and central field, may be that, in the present VEP paradigm, the responses of the central retina are evaluated. In most patients, bleaching abnormalities may be best detected in the macular region, where accumulated lipofuscin can also be observed more frequently.[31,33] In addition, light-adapted, contrast-evoked responses may be specifically suited to reveal cone system abnormalities occurring after exposure to a bright-adapting light.

It is of interest to note that different clinical studies have reported abnormal recovery of visual sensitivity (either rod-[34] or cone-[12,16,35] mediated) after bleaching in early age-related macular degeneration. This disorder shares some similarities with STD/FF, although the hypothesis of a common genetic background for both diseases is controversial.[36,37] The present findings could provide a further link between the two disorders and suggest the use of the VEP postbleaching test to characterize the functional status of age-related macular degeneration in patients.

In conclusion, the present data indicate abnormalities in the recovery of macular function after bleaching in STD/FF. These abnormalities are associated with the severity of outer retinal dysfunction, as measured in the baseline condition by the FERG. The results, pointing toward altered macular cone photoreceptor adaptation at a stage of the disease with still normal visual acuity, may be relevant for a better understanding of STD/FF pathophysiology.

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